ORIGINAL ARTICLE

Pedicled Bone Flap Formation Using Transplanted Bone Marrow Stromal Cells

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Hypothesis: Transplanted osteoprogenitor cells derived from cultured bone marrow stromal cells (BMSCs) can be used to fabricate pedicled bone flaps.

Design: Prospective, randomized experimental trials.

Setting: Basic science research laboratory.

Materials: Immunodeficient female NIH-Bg-Nu-Xid mice, aged 3 months.

Intervention: The BMSCs were harvested from the long bones of C57Bl/6 transgenic mice carrying the type Iα(1) collagen–chloramphenicol acetyl transferase reporter gene construct; their numbers were expanded in tissue culture. Treated mice received BMSC transplantations around the common carotid artery and internal jugular vein, the aorta and its venae comitantes, or the saphenous artery and vein; control mice received a sham transplant in comparable recipient sites.

Main Outcome Measures: Mice underwent harvesting from 4 weeks to 2 years after transplantation. Transplants were evaluated via histological, immunohistochemical, and angiographic analyses.

Results: Compared with the controls, which formed no bone, 32 of 37 BMSC-containing transplants formed a vascularized bone island that was perfused specifically and solely by its common carotid artery vascular source. Mature transplants consisted of well-developed lamellar, corticocancellous bone whose osteocytes were derived from the grafted BMSCs; hematopoietic tissue derived from the recipient mouse. Transplants formed as early as 4 weeks and remained stable in size as late as 108 weeks.

Conclusions: Bone marrow stromal cells can be used to create vascularized bone flaps in mice; these bone constructs are vascularized by their pedicle and therefore can potentially be transferred to a recipient site using microsurgical techniques. These findings provide proof of principle of an additional clinical application of BMSC transplantation techniques.

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FRIEDENSTEIN and Owen[1,2] demonstrated the presence of a population of bone marrow stromal cells (BMSCs) with a pluripotent capability. These cells could be distinguished from most hematopoietic elements in the marrow by their high adherence to the substrate plastic in tissue culture flasks and by a number of morphologic, histochemical, and biochemical characteristics. Phenotypically, BMSCs have been found to be capable of differentiating into bone, cartilage, muscle, and adipose and neural tissue elements.[1-6] Populations of BMSCs that include osteoprogenitor cells have been expanded in tissue culture and transplanted into recipient animals. Such heterotopic transplants, whose donor BMSCs have been isolated from species ranging from rodents to humans, form bone in mouse recipients.[1,7-13] Bone marrow stromal cells also have been shown to repair induced bone defects in various animal models.[14,15]

Successful repair of bone defects, whether arising from trauma, tumor resection, or congenital disorders, continues to be a major concern to reconstructive surgeons.[16] Free flaps are often the only viable option when the recipient site is scarred by infection or radiation injury; however, their use is associated with greater risk to and discomfort for the patient.[16-19] In an effort to avoid the problems associated with standard bone-flap harvests, investigators have sought to fabricate flaps. Prefabricated flaps, whether used to reconstruct bone or soft tissue, can be created in an anatomic location that minimizes donor site morbidity. They can be designed to fit a particular recipient site, since their size and shape is not limited to those found naturally. Techniques for flap fabrication that are already in clinical

MATERIALS AND METHODS

PREPARATION OF MOUSE BMSCs

Bone marrow cells were harvested from the long bones of 8-week-old C57Bl/6 transgenic mice carrying the type Iα(1) collagen-CAT construct[25] using a technique described elsewhere.[26] All studies were completed in accordance with an approved small animal protocol (National Institutes of Health protocol 97-024). Briefly, mice were killed humanely with inhaled carbon dioxide; the femurs, tibias, and humeri were removed and cleaned of all adjacent soft tissue. The epiphyses were removed, and the bone marrow from each medullary cavity was flushed with α-minimal essential media (αMEM; Gibco, Grand Island, NY). Bone marrow cells from 6 long bones (6×10^7 to 8×10^7 nucleated cells) were plated to a single culture flask (T-75; Becton Dickinson; Lincoln Park, NJ) in complete medium consisting of αMEM, 2-mmol/L L-glutamine, 100-U/mL penicillin, 100-µg/mL streptomycin sulfate (Biofluids, Rockville, Md), 10^{-8}-mol/L dexamethasone (Sigma-Aldrich Corp, St Louis, Mo), 10^{-4}-mol/L L-ascorbic acid phosphate magnesium salt *n*-hydrate (Wako, Osaka, Japan), and preselected lots of 20% fetal bovine serum (Atlanta Biologicals, Atlanta, Ga). The cells were incubated at 37°C in 5% carbon dioxide. The medium was changed completely on day 2. When the adherent cell layer had reached confluence, typically at day 10 to day 12, the cells were rinsed with Hanks balanced salt solution (Gibco), detached with 2 portions of 1× trypsin-EDTA (Gibco), and again plated at 1:3 dilution in new flasks. Subsequent passages were performed in a similar manner.

TRANSPLANT PREPARATION AND ANIMAL OPERATIONS

After 2 to 6 passages, cells were released from tissue culture flasks by treatment with trypsin, and they were were prepared for transplantation by pelleting at 1000 rpm and resuspending in a small volume of complete medium. From 2×10^6 to 5×10^6 BMSCs were absorbed into sponges of cross-linked porcine collagen type I (Gelfoam; Upjohn, Kalamazoo, Mich) measuring 125 mm^3. The sponges were incubated at 37°C for 60 minutes. A control group of sponges was prepared under similar conditions; these were moistened with full medium but received no cells. Three-month-old immunodeficient NIH-Bg-Nu-Xid female mice (Harlan-Sprague Dawley, Indianapolis, Ind) served as transplant recipients. Mice were anesthetized using a combination of intraperitoneal ketamine hydrochloride (Fort Dodge Animal Health; Fort Dodge, Iowa) at 140 mg/kg body weight and intraperitoneal xylazine hydrochloride (Butler, Columbus, Ohio) at 7 mg/kg body weight. The bilateral common carotid arteries, saphenous vessels, or abdominal aorta were exposed. The arteries and their adjacent veins were isolated and encircled with the graft. The grafts were bilayered constructs. The inner layer, closest to the vessels, consisted of a sponge that did or did not include cells. The sponge was encircled by a strip of polytetrafluoroethylene (PTFE) (Gore-Tex; WL Gore & Associates; Flagstaff, Ariz) vascular graft material, constituting the graft's outer layer (**Figure 1**). The PTFE served to prevent vascularization of the transplant by the surrounding soft tissues. The incisions were closed in layers.

In an effort to confirm that the pedicle is the source of any vascularization of the transplants, some BMSC transplants (a small subset) were wrapped around pedicles that had first been encircled by an impermeable plastic membrane. This membrane served to prevent all vascularization

use have resulted in reconstruction of the calvaria, maxilla, esophagus, and penis.[20-24]

The aim of this study was to demonstrate the feasibility of creating vascularized bone islands using transplanted BMSCs. Bone marrow stromal cells were harvested from transgenic mice containing a type Iα(1) collagen–chloramphenicol acetyl transferase (CAT) reporter gene construct. Mouse BMSCs expressing CAT were expanded in tissue culture, loaded onto matrices, and transplanted into recipient mice around the paired common carotid artery and internal jugular vein. The establishment of new bone was confirmed using results of histological evaluation and plain radiography. Origin of osteoblasts within the transplants was demonstrated using immunohistochemical identification of CAT expression. Arteriography and perfusion of the transplants with tracer dyes was performed to demonstrate their vascularization. To confirm the importance of the pedicle to bone formation, BMSCs were transplanted around pedicles that had first been encircled by nonpermeable plastic membranes that impeded vascularization. Similar vascularized bone models were established using the aorta and the saphenous artery in the mouse. We also completed isogenic transplantations using BALB/c mice as BMSC donors and transplant recipients to demonstrate the feasibility of transplanting BMSCs to mice with intact immune systems.

RESULTS

A total of 48 transplants were delivered to 28 recipient mice. Five of the mice received BALB/c isografts, whereas the remainder were immunodeficient mice receiving transplants of BMSCs derived from C57Bl/6 transgenic mice harboring the type Iα(1) collagen-CAT 3.6 construct. Transplants were harvested from 4 to 108 weeks postoperatively. Thirty-two of 37 transplants with BMSCs formed bone, while all 11 cell-free transplants formed only fibrous connective tissue (**Figure 2**). Bone was formed as early as 4 weeks after transplantation and persisted, with no signs of degeneration, senescence, or sarcomatous transformation in transplants harvested at 108 weeks (**Figure 3**). Transplants free of BMSCs were harvested at 4, 8, 92, and 100 weeks; bone formation was equally absent at all 4 time points.

TRANSPLANT MORPHOLOGIC FEATURES

Transplants that had received cells from C57Bl/6 transgenic mice had characteristic morphologic features, of the transplant by the pedicle. The PTFE outer sheath served to prevent vascularization from the surrounding soft tissues.

To demonstrate that BMSC-mediated bone formation is not restricted to NIH-Bg-Nu-Xid mice, BMSCs were also transplanted into BALB/c mice with intact immune systems. Bone marrow was harvested from the long bones of BALB/c mice and cultured in a manner similar to cells from the transgenic mice. Syngeneic transplants made from these cells were placed in inbred BALB/c mice in the same fashion as had been done with transplants in the NIH-Bg-Nu-Xid mice.

EVALUATION OF BMSC TRANSPLANT RECIPIENTS

The mice and their transplants were evaluated using several techniques. To confirm that bone had formed in the transplants, recipient mice underwent radiography (Faxitron MX-20 Specimen Radiography System; Faxitron X-ray Corporation, Wheeling, Ill) at an energy of 30 kV for an exposure duration of 90 seconds (Kodak X-OMAT TL film; Eastman Kodak Company, Rochester, NY). In an effort to confirm pedicle patency and its contribution to transplant vascularization, mice underwent angiography. The mice were injected systemically via the left heart with sodium nitroprusside (Nipride; Gensia Laboratories, Inc, Irvine, Calif) and ethiodized oil (Ethiodol; Savage Laboratories, Melville, NY), then underwent radiography. To further confirm that the transplants were perfused by the vascular pedicle, the transplants were dissected free of surrounding soft tissue, remaining attached to the mice solely via the vascular pedicle. The mice were systemically injected with methylene blue via the left cardiac ventricle. The transplants, which were noted to immediately turn blue, were harvested, subjected to radiography, and processed for histological analysis.

TRANSPLANT ANALYSIS

Transplant recipients were killed humanely, and transplants were harvested from 4 to 108 weeks after transplantation. Following radiography, transplants were fixed and demineralized in Bouin fluid (Sigma-Aldrich Corp), embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

To confirm that osteoblasts and osteocytes within the transplants were of donor origin, immunohistochemical analysis for CAT was performed on the transplants. The transplants were fixed overnight at 4°C in 4% formalin in phosphate-buffered saline solution freshly prepared from paraformaldehyde; they were then decalcified with 10% EDTA (pH, 8.0) at 4°C for 2 days. The transplants were embedded (Tissue-Tek O.C.T. 4583 Compound; Sakura Finetechnical Co Ltd, Tokyo, Japan), frozen rapidly in ethanol–dry ice, and sectioned with a frozen microtome (Jung Frigocut; Leica Instruments GmbH, Wetzlar, Germany). Frozen sections of 7 to 10 μm in thickness were immersed in phosphate-buffered saline solution for 10 minutes. Endogenous peroxidase was inactivated by incubation with 3% hydrogen peroxide for 30 minutes. An avidin-biotinylated peroxidase complex was completed using rabbit anti–CAT antibody at a 1:500 dilution (5Prime-3Prime Inc, Boulder, Colo) as the primary antibody, and biotinylated anti–rabbit IgG antibody (Vector Laboratories, Burlingame, Calif) as the second antibody. The immunolocalized antigen was visualized by means of diaminobenzidine (HistoMark; Kirkegaard and Perry Laboratories, Gaithersburg, Md). Normal rabbit serum (1:500) and normal rabbit IgG (17 μg/mL) served as negative controls.

whether harvested early or late after transplantation (**Figure 4**A). The vascular pedicle lay at the heart of the transplant. It was encircled by 2 concentric layers of lamellar cortical bone; the first of these sat immediately adjacent to the pedicle, whereas the second lay further out against the inner wall of the PTFE sheath. Each layer of cortical bone was nearly intact, except for small and sporadic fenestrae. The space between the 2 layers of bone was filled with hematopoietic tissues and small trabecula-like bone islands (Figure 4B-C). Adipocytes were abundant. The amount of the newly formed bone and the size of the transplant ossicle remained constant from 8 weeks onward. In contrast, transplants that did not include cells formed no bone (Figure 4D). The space between the intact pedicle and the PTFE sheath was filled with a fibrocellular infiltrate; this included remnants of the original sponge carrier as late as 8 weeks but not beyond 12 weeks after transplantation (Figure 4E). The 5 BMSC-containing transplants that failed to form bone resembled histologically those transplants that had not received cells. They contained a mixture of residual sponge and fibrous connective tissue.

Radiographs of the animals were obtained as early as 4 weeks posttransplantation. Transplants containing cells were radiopaque and clearly distinct from surrounding structures, whether in the neck (**Figure 5**A), groin (Figure 5B), or abdomen. Radiographs of harvested transplants confirmed the presence of 2 concentric layers of cortical bone (Figure 5C).

DETERMINATION OF OSTEOCYTE ORIGIN

The BMSCs derived from transgenic mice carrying the 3.6 kilobase of the rat type Iα(1) collagen promoter fused to the CAT reporter gene were used to observe the fate of the transplanted cells. Expression of CAT served as a marker for donor cell activity because it is not present in the recipient cells. Tissue sections from a 4-week-old transplant were evaluated using an antibody raised against CAT. Expression of CAT was detected in osteoblasts and osteocytes within the cortical and trabecular components of the new bone, confirming that the osteogenic cells were of donor origin rather than originating from the local microenvironment (Figure 4F). Immunoreactivity of CAT was restricted to the new bone and was not present in the peritransplant tissues, suggesting that the BMSCs did not migrate outside the confines of the transplant. No signs of inflammation or dysplasia were detected in the peritransplant region of any of the implants analyzed.

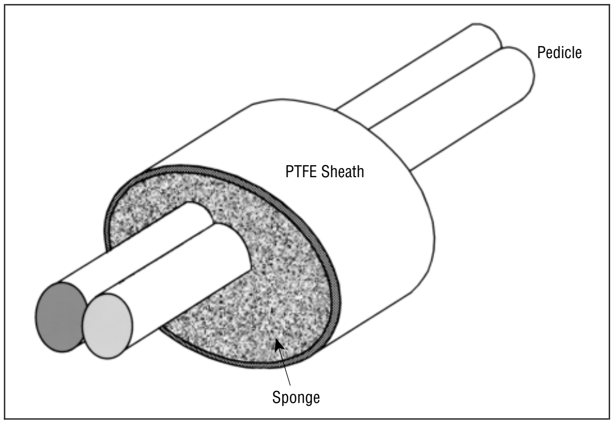

Figure 1. *Schematic diagram of transplants consisting of bone marrow stromal cells and sponges of cross-linked porcine type I collagen (Gelfoam; Upjohn, Kalamazoo, Mich). PTFE indicates polytetrafluoroethylene.*

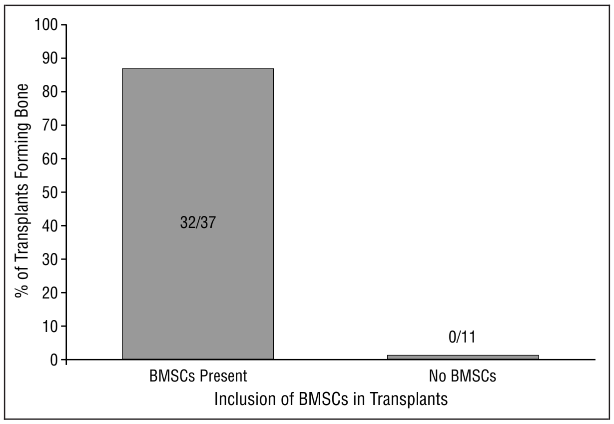

Figure 2. *Prevalence of bone formation among all pedicled transplants in all sites among NIH-Bg-Nu-XID and BALB/c mice. BMSC indicates bone marrow stromal cell.*

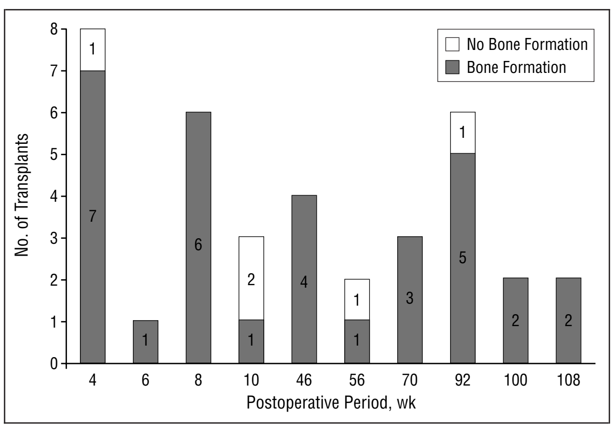

Figure 3. *Prevalence of bone formation at specific time intervals among all pedicled transplants that had received bone marrow stromal cells among NIH-Bg-Nu-XID and BALB/c mice.*

CONFIRMATION OF TRANSPLANT PERFUSION

Mice that had undergone systemic injection with ethiodized oil before radiography demonstrated the presence of radiopaque dye within the pedicle on coronal and axial views (Figure 5D-E). Transplants from mice that had received a systemic arterial injection of methylene blue before humane killing were examined histologically. These implants demonstrated a blue coloration arising from the arterially delivered dye. Similarly, gross visual examination of the in situ transplants confirmed the absence of any other vascular connections to the peritransplant tissues.

Transplants containing an impermeable plastic membrane immediately encircling the vascular pedicle were evaluated at 59 (1 transplant) and 70 (2 transplants) weeks. These transplants were prevented from establishing vascular connections to the pedicle or to surrounding soft tissues because of the plastic membrane and the PTFE, respectively. All 3 failed to form bone. Only minimal soft tissue remained of the original transplant, without any evidence of a mineralized structure.

BONE FORMATION IN ISOGENIC TRANSPLANTS

Isogenic transplants were harvested at intervals of 4 (1 transplant), 46 (3 transplants), 56 (1 transplant), and 70 (3 transplants) weeks. Seven of the 8 transplants formed bone; only the 4-week-old transplant failed. The transplant shape, size, and bone morphologic features were comparable to transplants derived from C57Bl/6 transgenic mouse cells that had been placed in immunocompromised recipients.

BONE FORMATION AT OTHER VASCULAR SITES

Bone formed among 7 of the 9 cell-containing transplants at the saphenous artery and 2 of the 3 cell-containing aortic transplants (**Figure 6**). Bone in these sites was comparable in shape, size, and morphologic features to that found in neck-based transplants.

COMMENT

Transplanted BMSCs have been shown to repair calvarial defects in mice and femoral defects in rats and dogs.[14,15,27] Such transplants have depended on a hospitable recipient bed, undamaged by scarring, radiation, or infection. Transplantation of BMSCs into an inhospitable location may require the transfer of a vascularized construct. In this study, we have developed a new model combining the bone-forming ability of culture-expanded BMSCs with prefabrication of a vascularized bone flap. We transplanted CAT-expressing cultured mouse BMSCs into immunosuppressed recipient mice around a vascular pedicle. Pedicles included the paired common carotid artery and internal jugular vein, the saphenous artery and vein, and the aorta and its venae comitantes. From periods of 4 to 108 weeks after transplantation, mice underwent histological, plain radiographic, and angiographic evaluation. Our data demonstrate that BMSCs can form bone that is perfused by a specific vascular pedicle. Perfusion was confirmed using arteriography and tracer dye injection. Interposition of an impermeable membrane between the cells and the pedicle at the time of transplantation resulted in no bone formation, further establishing the importance of the pedicle to vascularization and bone formation. Mature transplants were characterized by a double layer of cortical

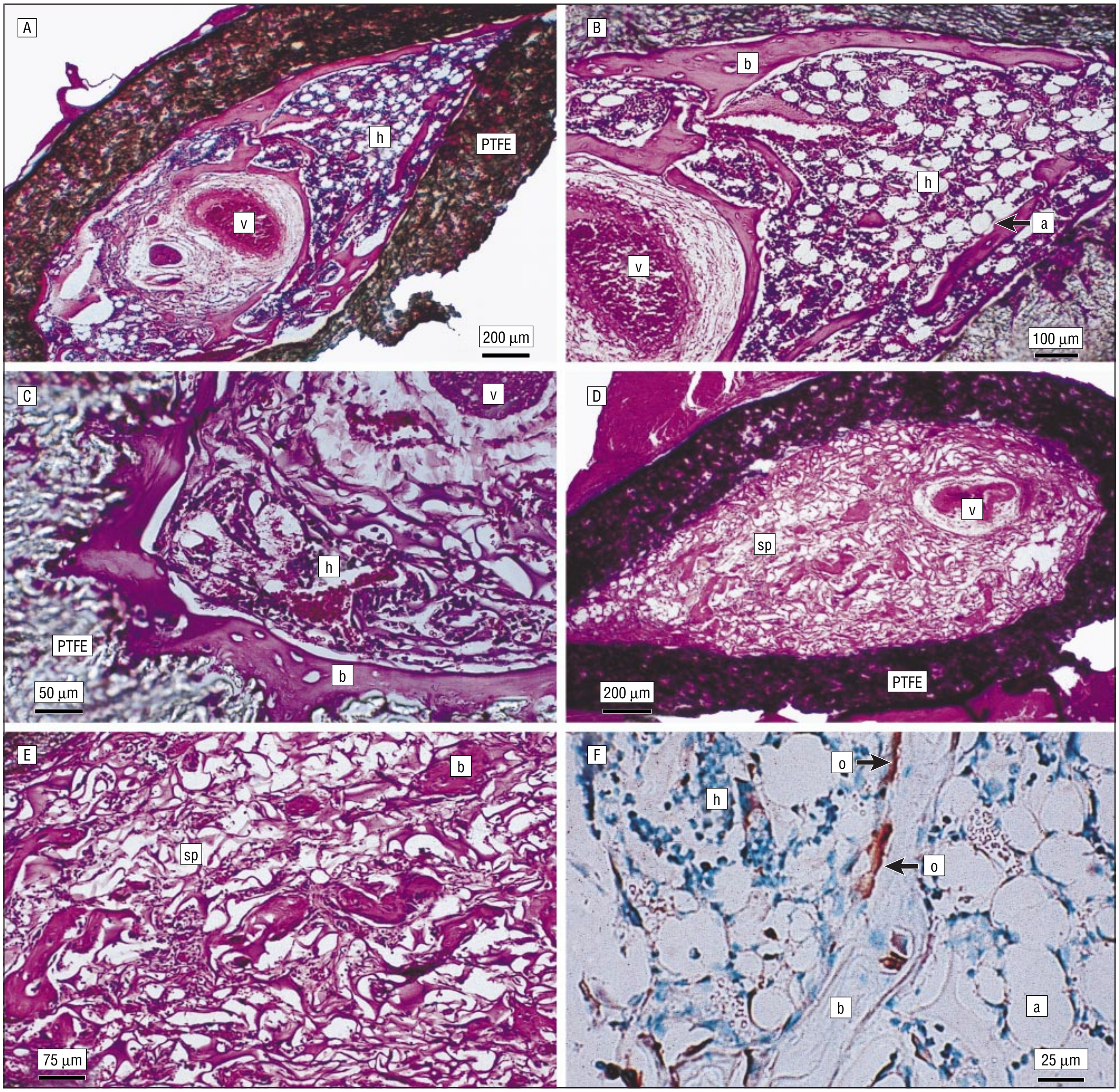

Figure 4. *Histological sections of bone marrow stromal cell (BMSC)–free or BMSC-containing transplants. A, Mature, 8-week-old BMSC-containing transplant that has formed corticocancellous bone (hematoxylin-eosin). B, Higher-power view of part A (hematoxylin-eosin). C, High-power view of 8-week-old BMSC-containing transplant. New cortical bone conforms to the silhouette of the polytetrafluoroethylene (PTFE) sheath (hematoxylin-eosin). D, Mature, 8-week-old BMSC-free transplant that has formed no corticocancellous bone (hematoxylin-eosin). E, Higher-power view of part D. Residual sponge (sp) is evident (hematoxylin-eosin). F, Confirmation of the donor origin of the newly formed bone in an 8-week-old BMSC-containing transplant. Immunoreactivity to type Iα(1) collagen–chloramphenicol acetyl transferase is localized to osteocytes in the new bone flap and is absent in the hematopoietic tissues (h). B indicates bone; v, vascular bundle; a, adipocytes; and o, osteocytes.*

bone, the outer layer forming the outer layer of the transplant, the inner layer surrounding the vascular pedicle, and the intervening space filled with hematopoietic tissue. The donor origin of the cells forming new bone was established using immunohistochemistry, with an antibody to CAT; in contrast, hematopoietic cells in such transplants are of recipient origin.[7] The persistence of bone in the latest time point (108 weeks) confirmed the stability of these transplants over time; in fact, the transplants maintained their shape and size despite the absence of mechanical stimuli of remodeling. We also demonstrated the feasibility of forming bone in mice with intact immune systems by using syngeneic transplants.

Of special interest is the difference in morphologic features between these pedicle-associated constructs and that of subcutaneous mouse BMSC transplants that are not associated with discrete vessels. The subcutaneous transplants consist of a shell of cortical bone that surrounds a space filled with hematopoietic elements and trabecular bone.[7] Such transplants do not have the inner layer of cortical bone that typifies the pedicled constructs. This inner cortical shell, and the outer as well,

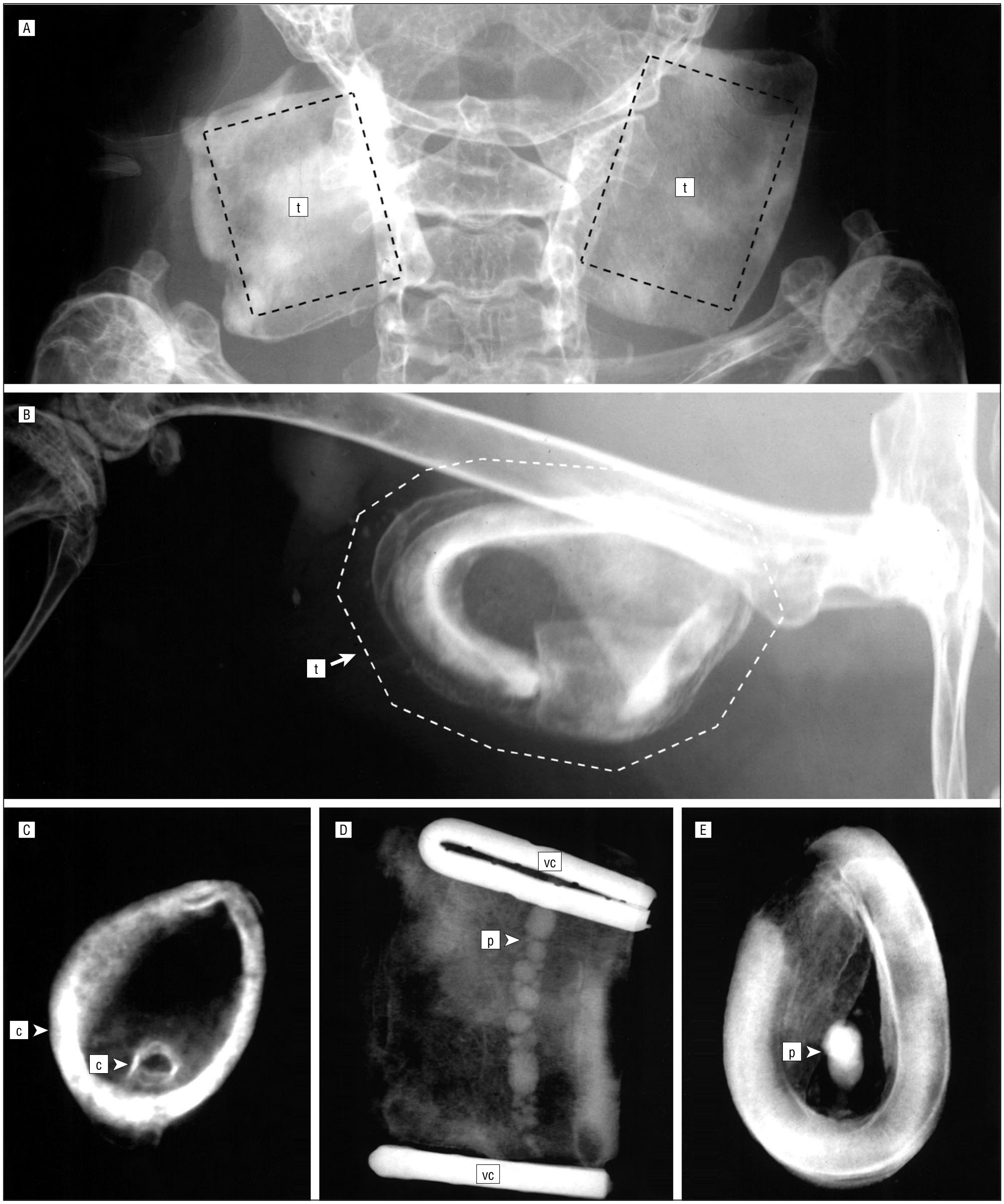

Figure 5. *Radiographs of bone marrow stromal cell (BMSC)–containing transplants. A, Anteroposterior view of mouse neck; 8-week-old transplants encircle the common carotid arteries and internal jugular veins. B, Anteroposterior view of mouse thigh; the 8-week-old transplant encircles the saphenous vascular bundle (arrow). C, Axial view of harvested 8-week-old transplant en face. Two concentric layers of cortical bone (arrowheads) are seen. Inner cortical shell encircles vascular bundle. D, Angiogram of a 108-week-old transplant, filmed after systemic arterial injection of ethiodized oil and harvesting of transplant. Vascular clips at either end of transplant maintain the dye in the intravascular space. Pedicle (arrowhead) remains patent despite the longevity of the transplant. E, Axial angiogram of 108-week-old transplant. Pedicle (arrowhead) is discretely opaque. t indicates transplant; vc, vascular clip; p, pedicle; and c, cortical bone layer.*

may arise in those portions of the transplant in greatest proximity to regions of higher oxygen tension or nutrient load. Alternatively, cortical bone may develop in those portions of the transplant that are immediately adjacent to host tissue structures; these structures may offer directionality to the osteogenic cells. Developing a greater understanding of this behavior may aid in developing bone constructs with a higher density of cortical elements and improved biomechanical characteristics.

Prefabrication of vascularized bone constructs is a steadily expanding field of investigation, although most studies involve preparation of the new bone using corticocancellous grafts, bone morphogenetic protein–2, or biocompatible ceramics.[28-30] Also, the transplantation of BMSCs and ceramic blocks into mouse latissimus dorsi muscle has been described.[31] The muscle, which provided a vascular-rich environment for bone formation, could be detached and transferred to another anatomic site, carrying the new bone construct along with it. In contrast, our model does not rely on muscle, but instead depends on a paired, expendable artery and vein. Optimum vessels, such as the superficial epigastric, would be superficial and easily dissected with minimal scarring of the patient; they could be moved without the patient losing strength or mobility. As well, our use of hydroxyapatite/tricalcium phosphate particles is an advantage over the use of the blocks; in conjunction with BMSCs in a mouse subcutaneous site, the particles have been associated with more extensive bone formation than the blocks.[7]

The system presented herein is clinically beneficial because it may obviate the need to harvest the vascularized fibula or iliac crest in select patients undergoing a bone reconstruction. A patient undergoing a staged reconstruction of the mandible in an irradiated site, for instance, could undergo a bone marrow harvest, ex vivo BMSC expansion, and establishment of an engineered bone flap in the groin. These relatively quick procedures would expose the patient to minimal morbidity. The flap could then be transferred from groin to face in the same way that a standard bone flap is handled. The remaining challenge, that of creating large-volume BMSCs transplants, is being addressed steadily. Cylindrical BMSC transplants 20 mm in length and 13 mm in diameter have formed bone in dogs, relying solely on peripherally based vascularization.[32] If ready vascularization is the limiting factor in transplant size, the use of exogenously administered angiogenic factors may allow for larger transplants.

This system could theoretically be used as an in situ biogenerator. Autogenous, culture-expanded BMSCs that have been engineered to express a specific product could be transplanted around a pedicle. The factors they express could directly enter the systemic circulation. In addition, the transplant could be removed if the factor was no longer required by the patient. Efforts are already under way to transfect human BMSCs to express factor IX, although to date none of these efforts have resulted in long-term expression.

In summary, we have created a new model that combines the bone-forming ability of culture-expanded BMSCs with surgical prefabrication techniques. The successful formation of such a vascularized bone flap offers new clinical and research opportunities. Relying on an expendable vascular pedicle, such transplants in patients could offer a method for obtaining vascularized autogenous bone without sacrificing the traditional donor sites. Alternatively, this technique may increase the feasibility of using transplanted BMSCs as autogenous biogenerators.

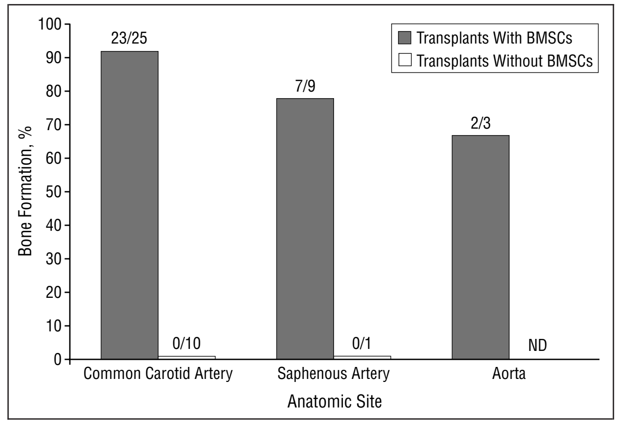

Figure 6. *Prevalence of bone formation by vascular site among all mice. BMSC indicates bone marrow stromal cell; ND, not done.*

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An Overview of the 4 Randomized Trials of Aspirin Therapy in the Primary Prevention of Vascular Disease

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Background: In the primary prevention of cardiovascular disease, in contrast to the recommendations of the American College of Chest Physicians and the American Heart Association, the US Food and Drug Administration recently stated that there was insufficient evidence to judge whether aspirin therapy decreases the risk of a first myocardial infarction.

Objective: To perform an overview of the 4 primary prevention trials of aspirin therapy to obtain the most reliable estimates of the effects of aspirin therapy on various vascular disease end points.

Methods and Results: These 4 trials included more than 51000 subjects and 2284 important vascular events. Those assigned to aspirin therapy experienced significant reductions of 32% (95% confidence interval [CI], 21%-41%) for nonfatal myocardial infarction and 13% (95% CI, 5%-19%) for any important vascular event. There were possible small but nonsignificant increases in risks of vascular disease–related death (1%; 95% CI, −12% to 16%) and nonfatal stroke (8%; 95% CI, −12% to 33%). When strokes were subdivided by type, there was no significant effect of aspirin therapy on the risk of ischemic stroke, but, while based on small numbers, there was a 1.7-fold apparent increase (95% CI, 6%-269%) in the risk of hemorrhagic stroke, which did achieve statistical significance.

Conclusions: For the primary prevention of vascular disease, aspirin therapy confers significant beneficial effects on first myocardial infarction and, as a result, on any important vascular event; these effects are clinically important. Whether there is any reduction in vascular disease–related death or stroke associated with treatment remains unclear because of inadequate numbers of events in the primary prevention trials completed to date. More data on hemorrhagic stroke are also needed. In addition, randomized trial data, especially in women but also in men, are needed to help to formulate a rational public health policy for individuals at usual risk. Meanwhile, these data provide evidence for a significant benefit of aspirin therapy in the primary prevention of myocardial infarction. (*2000;160:3123-3127*)

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